The Antimicrobial Resistance Crisis: The Urgent Need for Alternative Therapies

Michael J. Rybak, PharmD, MPH, PhD
Professor of Pharmacy
Eugene Applebaum College of Pharmacy & Health Sciences
Adjunct Professor of Medicine, Division of Infectious Diseases
School of Medicine,
Wayne State University
Eugene Applebaum College of Pharmacy & Health Sciences

• 11 accredited healthcare disciplines
  • Offers degrees or certificates to undergraduates, professional and graduate students
• Research: Cancer, Infectious Diseases, Metabolic, Neuro/Psyc

• BS: Clinical Laboratory Science, Mortuary Science, Radiologic Technology, Radiation Therapy Technology, Health Sciences
• MS: Pathologists Assistant, Physician Assistant, Pharmaceutical Sciences, Occupational Therapy
• Doctorates: Physical Therapy, Nurse Anesthesia, Pharmacy (PharmD), PhD Pharmaceutical Sciences
Background

- Born and raised in Buffalo NY - 🏈
- Pre-Medicine Daeman University
  - Roswell Park Cancer Institute
- Associate of Science Degree
- Buffalo State University - Biology
- Northeastern University, Boston MA
  - B.S. Pharm -1979
- Hospital Pharmacist
  - South Shore Hospital – South Weymouth MA
- Wayne State University
  - PharmD -1981
- Faculty Position WSU 1981
  - DMC appointment
    - Clinical Pharmacokinetic Service
    - Pharmacokinetics Laboratory
- Microbiology/Antibiotic Resistance
  - Glenn Kaatz, MD
  - Sabbatical –Antibiotic Resistance
- Fellowship Program- 1985
- Focus
  - Antibiotic optimization and prevention of antibiotic resistance
- Associate Professor-Tenure - 1987
- Professor-Tenure -1993
- Associate Dean for Research – 2003-2011
- MPH WSU – 2005
- PhD Walden University Public Health/Epidemiology 2016
Vancomycin Pharmacokinetics in Burn Patients and Intravenous Drug Abusers
MICHAEL J. RYBAK,1,2*, LISA M. ALBRECHT,1†, JULIE R. BERMAN,2 LAWRENCE H. WARBASSE,3,4 and CRAIG K. SVENSSON1

College of Pharmacy and Allied Health Professions1* and School of Medicine,2 Wayne State University, Detroit, Michigan 48202, and Departments of Pharmacy Services2 and Internal Medicine,4 Detroit Receiving Hospital and University Health Center, Detroit, Michigan 48201

Teicoplanin Pharmacokinetics in Intravenous Drug Abusers Being Treated for Bacterial Endocarditis
MICHAEL J. RYBAK,1,2,5,6,7 STEPHEN A. LERNER,7,8 DONALD P. LEVINE,2,5,6,7 LISA M. ALBRECHT,1,9† PAM L. MCNEIL,5,6,7 GARY A. THOMPSON,5,6,7 MICHAEL T. KENNY,7 and LIANG SHI YU8

College of Pharmacy and Allied Health Professions1* and School of Medicine,2 Wayne State University, Departments of Pharmacy3 and Internal Medicine,4 Detroit Receiving Hospital and University Health Center,5 and Division of Infectious Diseases, Department of Medicine,6 Harper Hospital,7 Detroit, Michigan 48201; Merrell Dow Research Institute,8 Cincinnati, Ohio 45215; and Merrell Dow Research Institute, Indianapolis, Indiana 46268

Prospective Evaluation of the Effect of an Aminoglycoside Dosing Regimen on Rates of Observed Nephrotoxicity and Ototoxicity
Michael J. Rybak1,2,†, Betty J. Abate3,†, S. Lena Kang4,†, Michael J. Ruffing1, Stephen A. Lerner2, and George L. Drusano5
1 The Anti-Infective Research Laboratory, Department of Pharmacy Services, Detroit Receiving Hospital and University Health Center, College of Pharmacy and Allied Health Professions, and
2 Department of Internal Medicine, Division of Infectious Diseases, School of Medicine, Wayne State University, Detroit, Michigan 48201, and
3 Division of Clinical Pharmacology, Departments of Medicine and Pharmacology, Albany Medical College, Albany, New York 12208

Pharmacokinetics and Bactericidal Rates of Daptomycin and Vancomycin in Intravenous Drug Abusers Being Treated for Gram-Positive Endocarditis and Bacteremia
MICHAEL J. RYBAK,1,2,5,6 ELAINE M. BAILEY,1,2,3 KENNETH C. LAMP,1,3,† and GLENN W. KAATZ1,4

College of Pharmacy and Allied Health Professions1* and Department of Medicine, Division of Infectious Diseases,1 Wayne State University, and the Anti-Infective Research Laboratory, Department of Pharmacy,5 and Department of Medicine,6 Detroit Receiving Hospital and University Health Center, Detroit, Michigan 48201

Pharmacodynamic Characterization of Nephrotoxicity Associated with Once-Daily Aminoglycoside
Kellie R. Murry, Pharm.D., Peggy S. McKinnon, Pharm.D., Beatriz Mitrzyk, Pharm.D., and Michael J. Rybak, Pharm.D., FCCP


Clinical use and toxicity of high-dose tobramycin in patients with pseudomonal endocarditis
Michael J. Rybak*, Steven C. Boike*, Donald P. Levine†, and Steven R. Erickson*

Department of Pharmacy* and the Division of Infectious Diseases, Department of Medicine,† Detroit Receiving Hospital and University Health Center, and Wayne State University, Detroit, Michigan, U.S.A.

Journal of Antimicrobial Chemotherapy. (1986) 17, 115-120.
Standard of care antimicrobials for serious MRSA infections

Vancomycin
Daptomycin
Ceftaroline

Vancomycin Therapeutic Guidelines: A Summary of Consensus Recommendations from the Infectious Diseases Society of America, the American Society of Health-System Pharmacists, and the Society of Infectious Diseases Pharmacists

Michael J. Rybak,1,2,10 Ben M. Lemon世家,11 John C. Rotschasche,12 Robert C. Meuller,1c William A. Craig,1
Marianne Billietto,1 Joseph R. Dalovisio,11,12 and Donald P. Levine1

1Azr Infectious Research Laboratory, Department of Pharmacy Practice, College of Pharmacy & Health Sciences, and Department of Medicine, School of Medicine, Wayne State University, and Detroit Receiving Hospital & University Health Center, Detroit, Michigan, 2Adıyaman Medical Center, Adıyaman, Turkey, 3Department of Experimental and Clinical Pharmacology, College of Pharmacy, University of Minnesota, Minneapolis. 4Shields Warren Malindroff Medical Research, Harvard Medical School, and Department of Medicine, Beth Israel Deaconess Medical Center, Boston, Massachusetts, 5University of Wisconsin School of Medicine and Public Health, Madison, and 6Children’s Medical Centers


Clinical Practice Guidelines by the Infectious Diseases Society of America for the Treatment of Methicillin-Resistant Staphylococcus aureus Infections in Adults and Children: Executive Summary

Catherine Liu,1 Arnold Bayer,1,2 Sara E. Cosgrove,1 Robert S. Dowell,1 Scott K. Fridkin,1 Rachel J. Gorwitz,1
Sheldon L. Kaplan,1,2,3 Adolf W. Karchmer,4 Donald P. Levine,11 Barbara E. Murray,5 Michael J. Rybak,6,12,13 David A. Talan,1,13 and Henry F. Chambers1,12

1Department of Medicine, Division of Infectious Diseases, University of California San Francisco, San Francisco, California. 2Division of Infectious Diseases, San Francisco General Hospital, San Francisco, CA. 3Division of Infectious Diseases, Hartford UConn Medical Center, Torrington, CA. 4Division of Emergency Medicine and Infectious Diseases, Drexel University College of Medicine, Philadelphia, PA. 5Department of Medicine, Danforth School of Medicine at University of California (Los Angeles). 6Division of Infectious Diseases, Johns Hopkins Medical Institutions, Baltimore, Maryland. 7Department of Pediatrics, Section of Infectious Diseases, University of Wisconsin, Madison, Madison, WI. 8Division of Healthcare Quality Promotion, Center for Prevention and Health Infection


Clinical Infectious Diseases

Therapeutic Monitoring of Vancomycin for Serious Methicillin-resistant Staphylococcus aureus Infections: A Revised Consensus Guideline and Review by the American Society of Health-system Pharmacists, the Infectious Diseases Society of America, the Pediatric Infectious Diseases Society, and the Society of Infectious Diseases Pharmacists

Michael J. Rybak,1,10 Jennifer Lu,1 Thomas P. Levine,11 Donald P. Levine,11 John S. Bradley,11 Catherine Liu,11 Bruce A. Meuller,11 Manjusha P. Pai,11 Annie Wong-Berenger,11 John C. Rotschasche,12 Keith A. Rodvidel,11 Holly D. Bogel,11 and Benjamin Lemon世家

1Azr Infectious Research Laboratory, Department of Pharmacy Practice, Eugene Applebaum College of Pharmacy and Health Sciences, Wayne State University, Detroit, Michigan, 2Adıyaman Medical Center, Adıyaman, Turkey, 3Department of Experimental and Clinical Pharmacology, College of Pharmacy, University of Minnesota, Minneapolis. 4Shields Warren Malindroff Medical Research, Harvard Medical School, and Department of Medicine, Beth Israel Deaconess Medical Center, Boston, Massachusetts, 5University of Wisconsin School of Medicine and Public Health, Madison, and 6Children’s Medical Centers


AHA Scientific Statement

Infected Endocarditis in Adults: Diagnosis, Antimicrobial Therapy, and Management of Complications

C. Kenneth Chuang, MD, FAHA; John J. Burns, MD, MSc; Michael J. Rybak, PharmD, MPH; Bruno Barsic, MD, PhD; Peter B. Luckhart, DDS; Michael H. Gewitz, MD, FAHA; Matthew E. Levison, MD; Ann F. Brolaz, MD, FAHA; James M. Stockelberg, MD; Robert S. Baltimore, MD; Anne M. Fink, PhD, RN; Patrick O’Gara, MD, FAHA; Kathryn A. Taubert, PhD, FAHA; on behalf of the American Heart Association Committee on Rheumatic Fever, Endocarditis, and Kawasaki Disease of the Council on Cardiovascular Disease in the Young, Council on Clinical Cardiology, Council on Cardiovascular Surgery and Anesthesia, and Stroke Council

Circulation. 2015;132:1435-86. doi: 10.1161/CIR.0000000000000296
Vancomycin

- Treatment of choice for MRSA Infections
  - In clinical use since 1958
- Mechanism of action
  - Cell wall synthesis inhibitor
- Resistance:
  - Low level: hVISA, VISA
  - High level: VRSA
  - Cross-resistance (i.e., daptomycin)
- Failure rates
  - High in complicated infections (i.e., BSI, IE)
  - 30-day mortality > 20% in cBSI

Rationale for Combination Therapy

Improved Patient Response
- Reduction in time to resolution of symptoms

Improved Drug Performance
- Potential for synergy
- Lower PK/PD target threshold
- Increased killing
- Decreased time to bacterial eradication

Lower Antibiotic Exposures
- Dose sparing
- Dose de-escalation
- Reduction of adverse effects

Reduction of the potential for Resistance
- Due to lower exposures
- Elimination/reduction of relapse and recurrence

Rodríguez-Gascón et al., 2021, Pharmaceutics 13:833
Combination Daptomycin or Vancomycin with Ceftaroline for Daptomycin and Vancomycin Non-susceptible S. aureus

Vancomycin Intermediate, Daptomycin Non-susceptible S. aureus: D712
(DAP MIC=4, CPT MIC=0.5, VAN MIC=4)

- GC
- DAP
- VAN
- CPT
- VAN + CPT
- DAP + CPT

Daptomycin 10 mg/kg/day
Vancomycin 2g q 12 h
Ceftaroline 600 mg q 8 h

CASE IN POINT:  Patient with persistent MRSA/VISA Bacteremia, Recalcitrant to Vancomycin or Daptomycin Therapy, Resolved Upon Addition of **Nafcillin**

Rapid MRSA Bacteremia Clearance with High-Dose Daptomycin plus a β-lactam

<table>
<thead>
<tr>
<th>MRSA Bacteremia</th>
<th>DAP Susceptible</th>
<th>VAN Failure</th>
<th>DAP 10 Failure</th>
<th>DAP 10+OXA Clear 24h</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>VAN Failure</td>
<td>DAP 10 Failure</td>
<td>DAP 10+NAF Clear 24h</td>
</tr>
<tr>
<td></td>
<td></td>
<td>VAN Failure</td>
<td>DAP 8 Failure</td>
<td>DAP 8+NAF Clear 24h</td>
</tr>
</tbody>
</table>

*Relapsed – 12 wks & 8 wks post-therapy – 1 cleared w/another course; 1 died w/VISA PV IE VAN MIC 3; DAP MIC 1.5 Red VISA; DAP MIC 2-4 - Additional studies performed on the isolates from this case

β-lactam adjuvant Therapy for MRSA Bacteremia: Translating bench to bedside

• Review of MRSA Bacteremia at the DMC
  • Up to 30% mortality
• MRSA Pathway- 2016
  • Based on laboratory experience with combination therapy
  • Published clinical studies

MRSA culture positive

Vancomycin 1st+Cefazolin
  • No vancomycin allergy
  • Not treated previously with vancomycin
  • Continue if clinically improved

Daptomycin 2nd +Cefazolin if vancomycin fails
  Daptomycin HD plus ceftaroline if daptomycin fails

Discontinue Cefazolin if negative culture 48-72 h

Combination Beta-Lactam Pathway for MRSA Bacteremia: STAPH Study

Phage Therapy: An adjunct to Antibiotic Treatment?

Michael J. Rybak, PharmD, MPH, PhD, FCCP, FIDSA, FIDP
Professor of Pharmacy,
Director, Anti-Infective Research Laboratory
Adjunct Professor of Medicine, Division of Infectious Diseases,
Wayne State University, Detroit MI. USA
Disclosures

• Received grant support, advisor or have spoken on behalf of:
  • Abbive, Amplify (now Armata), Contrafect, Entasis, La Jolla, Merck, Paratek, Shionogi, T2 Biosystems
  • NIH R21 AI163726 (PI)
  • NIH R01 AI1300056-04 (Co-Inv, A. Bayer, PI)
  • NIH R01 AI148342-03 (Co-Inv, C. Arias, PI)
  • Michigan Department of Health and Human Resources (PI)
Deaths Attributable to AMR Every Year

AMR in 2050
10 million

- Tetanus: 60,000
- Road traffic accidents: 1.2 million
- Cancer: 8.2 million
- Measles: 130,000
- Cholera: 100,000–120,000
- Diarrhoeal disease: 1.4 million
- Diabetes: 1.5 million

AMR now
700,000 (low estimate)


Two major goals of the National Action Plan (2020-2025) for combating AMR

Accelerate basic and applied research for development of novel therapeutics.

Slow the emergence of AMR bacteria and prevent their spread.

CDC

Urgent Threats

• Carbapenem-resistant *Acinetobacter*
• *Candida auris*
• *Clostridioides difficile*
• Carbapenem-resistant Enterobacteriacea
• Drug-resistant *Neisseria gonorrhoeae*

WHO

Panel: WHO priority list for research and development of new antibiotics for antibiotic-resistant bacteria

• Multidrug-resistant and extensively-resistant *Mycobacterium tuberculosis*

Other priority bacteria:
Priority 1: Critical
• *Acinetobacter baumannii*-carbapenem resistant
• *Pseudomonas aeruginosa*, carbapenem resistant
• Enterobacteriaceae, carbapenem resistant, third generation cephalosporin resistant

US Centers for Disease Control and Prevention 2019
WHO panel of 74 Experts: multi-drug resistant organisms requiring priority for discovery, research and development of new antibiotics.
What Role Could Phages play in the Treatment of Multi-drug Resistant Bacterial Pathogens?
What are Phages?

Viruses that infect bacteria

Nature’s “check on bacteria

Most abundant organism $10^{31}$

Highly specific

Harnesses as treatment For the last 100 years but not FDA approved

Good safety profile generally considered safe

Antibiotics vs. Bacteriophages

- Static molecules
- Broad host ranges
- Easier to commercialize
- Antimicrobial resistance challenging

- Dynamic, living organisms
- Extremely narrow host range
- Highly individualized
- High therapeutic Index
- Commercialization challenging
- Bacterial resistance to phage can be an issue
- Appears to be effective against biofilms
History of Phage Therapy

Phage Therapy in the Postantibiotic Era

1898 - Gamaleya “Ferments that can destroy bacteria”

1906 - Hankin - Antiseptic action of river water against Vibrio cholerae.

1915 - Twort - Bacteriolytic agents of enzymatic nature

1917 - d’Herelle - “Bacteria are susceptible to infection and are hosts to ultramicroscopic agents, named Bactériophages”

1919 - Birth of phage therapy. Dysentery cases cured using phage plaques

1929 - Fleming - First mention of penicillin

1930s-1939 - Ellis & Delbrück - Broad characterisation of the phage life cycle: adsorption, growth within host, lysis.

1937-1939 - Phage Therapy in the Postantibiotic Era

1940 - Ruska - First EM image of a phage

1942 - Introduction of penicillin

1940s-1970s - Golden Age of Antimicrobials Over 40 antibacterial compounds discovered and introduced.

- Beta-lactams
- Aminoglycosides
- Tetracyclins
- Macrolides
- Glycopeptides
- Lincosamides
- Sulphonamides
- Rifampicins

1977 - Sanger - First phage genome sequenced

1983-1986 - Slopek - Smith & Huggins - Phage therapy against antibiotic-resistant bacterial strains, in humans and multiple animal models. Infections including septicemia, and meningitis

1992-1996 - Soothill - Merrill et al. - Resurgence of phage therapy studies with animal models

2000 - Number of new antibiotics decreases. Start of the dry pipeline phenomenon

2006 - First commercial phage-based biocontrol product. For use against Listeria monocytogenes

2009 - Wright et al. - First phase I/II controlled clinical trial of phage therapy. Chronic otitis due to MDR-Pseudomonas aeruginosa

2015 - Phagoburn trial. Multicenter phase I/II. Wound infections in burned patients.

2017 - Schooley et al. - Successful personalised intravenous phage therapy. Acinetobacter baumannii septicemia

Phage Therapy: Commercial Cocktails for Empiric and Customized Treatment

- **Staphylococcal Bacteriophage**: S. aureus
- **PYO Bacteriophage**: S. aureus, E. coli, Streptococcus, Pseudomonas, Proteus
- **ENKO Bacteriophage**: Shigella, Salmonella, E.coli, Staphylococcus
- **INTESTI Bacteriophage**: Shigella, Salmonella, Staphylococcus spp. Proteus, E. coli, Pseudomonas aeruginosa, E. faecalis
- **SES Bacteriophage**: Staphylococcus, E. coli, Streptococcus
- **FERSISI Bacteriophage**: Staphylococcus, Streptococcus
- **Auto Bacteriophage**: customized “individual phage”
## Eliava Phage Therapy for Bacterial Persistence: Case Examples

<table>
<thead>
<tr>
<th></th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (yrs)</strong></td>
<td>43</td>
<td>64</td>
<td>72</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td>male</td>
<td>female</td>
<td>female</td>
</tr>
<tr>
<td><strong>Diagnosis</strong></td>
<td>Cystic fibrosis</td>
<td>Primary ciliary dyskinesia, bronchiectasis</td>
<td>Chronic cystitis, bacterial vaginitis</td>
</tr>
<tr>
<td><strong>Main Causative Pathogen</strong></td>
<td><em>P. aeruginosa</em></td>
<td><em>P. aeruginosa</em></td>
<td><em>K. pneumoniae</em></td>
</tr>
<tr>
<td><strong>Route of administration</strong></td>
<td>Oral, inhalation via nebulizer</td>
<td>Oral</td>
<td>Oral, vaginal suppositories</td>
</tr>
<tr>
<td><strong>Other phages included</strong></td>
<td>Custom PA, PYO, Intesti</td>
<td>Custom PA, Staph phage</td>
<td>Custom KP, Intesti, SES</td>
</tr>
<tr>
<td><strong>Antibiotics included</strong></td>
<td>None*</td>
<td>None</td>
<td>Vaginal suppositories: metronidazole, miconazole, polymyxin B/neomycin</td>
</tr>
<tr>
<td><strong>Phage duration of therapy</strong></td>
<td>Jan 2017-Feb 2021</td>
<td>Sept 2018-present</td>
<td>June 2018-June-2019</td>
</tr>
</tbody>
</table>

Personalized Phage Therapy for Disseminated MDR- *Acinetobacter baumannii* infection

- 2016 Egypt vacation
- MDR A. *baumannii* pancreatitis
- Univ California-San Diego
- Critical Condition-Comatose
- Phage cocktails-Texas A&M Univ, Dept of US Navy, Ampliphi
- Rapid response starting 48h post phage therapy
- 2019 Publication of “The Perfect Predator”

Lessons Learned From the First 10 Consecutive Cases of Intravenous Bacteriophage Therapy to Treat Multidrug-Resistant Bacterial Infections at a Single Center in the United States

Saima Aslam,1,2 Elizabeth Lampley,2 Darcy Wooten,1 Maile Karris,1 Constance Benson,1,2 Steffanie Strathdee,1,2 and Robert T. Schooley1,2

1Division of Infectious Diseases and Global Public Health, University of California, San Diego, La Jolla, California, USA, and 2Center for Innovative Phage Applications and Therapeutics, University of California, San Diego, La Jolla, California, USA

**Background.** Due to increasing multidrug-resistant (MDR) infections, there is an interest in assessing the use of bacteriophage therapy (BT) as an antibiotic alternative. After the first successful case of intravenous BT to treat a systemic MDR infection at our institution in 2017, the Center for Innovative Phage Applications and Therapeutics (IPATH) was created at the University of California, San Diego, in June 2018.
Considerations for the Use of Phage Therapy in Clinical Practice

Gina A. Suh, Thomas P. Lodise, Pranita D. Tamma, Jane M. Knisely, Jose Alexander, Saima Aslam, Karen D. Barton, Erica Bizzell, Katherine M. C. Totten, Joseph L. Campbell, Benjamin K. Chan, Scott A. Cunningham, Katherine E. Goodman, Keryll E. Greenwood-Quaintance, Anthony D. Harris, Shayla Hesse, Anthony Marasco, Veronique Nussenblatt, David Pride, Michael J. Rybak, Zoe Sund, David van Duin, Daria Van Tyne, Robin Patel, for the Antibacterial Resistance Leadership Group

- **Review:**
  - 2000-August 2021
  - English-language only
  - Reviewed:
    - 14,841 abstracts
    - 968 manuscripts
  - **65 cases total**
  - Age: 2-88 years
  - Female: 17; 26%, Male: 44; 68%
  - Unknown 4; 6%

**Targeted Organisms:**
- *P. aeruginosa* - 22
- *S. aureus* - 22
- *Acinetobacter* - 7
- Polymicrobial - 7
- *K. pneumoniae* - 6
- *S. epidermidis* - 3
- *Achromobacter* - 2
- *E. coli* - 2
- *M. abscessus* - 2
- *Burkholderia dolosa* - 1
- *E. faecalis* - 1
- *E. faecium* - 1
- GBS - 1
Phage Therapy Knowledge Gaps

• Infection types
• Efficacy: alone, + antibiotics
• Safety
• Antibiotics combination
  • Synergy, additive, antagonism
• PK/PD optimization
• Dosing/frequency/route/duration
  • Concurrent with antibiotic/sequential?
• Immune system impact

Phage-Antibiotic Combinations
Preserving Antibiotics Through “Smart Design”

Adjuvant Antibiotics

Phage-antibiotic synergy

Antibiotic “resensitization”

Phage Cocktails

Broader spectrum of activity

Circumnavigate phage resistance
Bacteriophage Therapy: Two approaches

- Isolate active phages
- Specialty genes checkpoint
- Propagation and purification
- FDA approval

Individualized Approach
- Patient receives targeted phage therapy
- Time frame weeks to months!

Commercial Approach
- Screen for broad activity ➔ Test with antibiotics ➔ Propagate/purification ➔ Animal/Clinical Trials
- FDA approval
**Bacteriophage AB-SA01 Cocktail in Combination with Antibiotics against MRSA-VISA in an Ex-vivo SEV PK/PD model**

- **Evaluated AB-SA-01**
  - Consist of 3 myoviruses related to Staphylococcus phage K
    - Sa83, Sa87 and J-Sa36
    - 1.5 x 10^8 PFU/ml
  - **MRSA**
    - D712 (DNS-VISA, agr2, USA100, ST-5)
      - MICs: DAP=4, VAN=4, CFZ>64, CPT=0.5 mg/L
  - **Time-kill analysis**
    - ½ MIC of antibiotics or peak conc. if resistant (CFZ)
    - Phage = 7.5 x 10^6 PFU/ml
    - Bactericidal > 3 and synergy > 2 log_{10} CFU/ml reduction

Developed an *Ex Vivo* simulated endocardial vegetation (SEV) model

- Proctor & Gamble Animal Alternative Research Grant

**SEVs**
- Consists of human fibrin, platelets, high bacterial burden and thrombin

**Glass Model Apparatus**
- Filled with media to support bacterial growth, sample ports to retrieve SEVs over time for bacterial quantification

**Computerized peristaltic pumps**
- Allows for simulation of humanized antibiotic pharmacokinetics

**Validated vs. 4 rabbit infective endocarditis models**
Ex-vivo SEV PK/PD Model

• Ex-vivo PK/PD model
  • Simulated endocardial vegetations
  • D712: $10^9 \log_{10} \text{CFU}/0.5\text{g SEV}$
  • Phage $1.5 \times 10^8 \text{PFU/ml q 12 h x 96 h}$

• Antibiotics
  • VAN 2 g q 12 h x 96h
  • CFZ 2 g q 8 h x 96h

Ex-Vivo PK/PD SEV Model: Results

Bacteriophages: *S. aureus, Enterococcus faecium, Pseudomonas aeruginosa*

**Collaborators**

- Susan Lehman, PhD
  - Center for Biologics Evaluation and Research, US FDA, Silver Spring, MD

- Biswajit Biswas, PhD, MS
  - Chief of Bacteriophage Science Division
  - Naval Medical Research Center, Fort Detrick, MD

- Breck A. Duerkop, PhD
  - Dept. Immunology and Microbiology, University of Colorado School of Medicine, Aurora, CO

- Jose Alexander, MD
  - Department of Microbiology, Virology and Immunology, AdventHealth Central Florida, Orlando, FL

- Rob Lavigne, PhD
  - Research and Development, Katholieke University, Leuven Belgium

- Razieh Kebriaei, PhD
  - Dept. Outcomes and Translational Sciences, The Ohio State University, Columbus, OH

- Cesar Arias, MD, PhD
  - Division of Infectious Diseases, Houston Methodist Hospital, Houston, TX

- Arnold Bayer, MD
  - The Geffen School of Medicine, UCLA, Los Angeles, CA

- Robert Bonomo, MD
  - Cleveland VA Medical Center, Case Western Reserve University, Cleveland, OH
Phage-antibiotic Co-therapy Composition Optimization against *S. aureus*

**Fig 1.** Plaque-based host range for 5 short-listed phages in our collection that gave the best coverage of the screened 72 strain library. Collectively, 69/72 (96%) of strains were sensitive to at least one of Sb-1, Intesti13, or Romulus. *LZD-R*=linezolid-resistant, *ST*=multilocus sequence type, *TZD-R*=tedizolid-resistant, *VISA*=vancomycin-intermediate *S. aureus.*

**Phages**
- *Herelleviridae* and *Twortvirinae* family
- K phage obtained from ATCC
- Sb-1 from Eliava Institute, Tbilisi, Georgia
- Stab21 isolated from Albania
- Romulus isolated in Belgium

Supported by NIAID R21 AI163726
Research in Progress
Phage Screening Genome Similarity

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<tr>
<th></th>
<th>Romulus (NC_020877)</th>
<th>Stab21 (LR215719*)</th>
<th>Sb-1 (NC_023009)</th>
<th>Intesti13</th>
<th>K (NC_005880*)</th>
</tr>
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<tbody>
<tr>
<td>Romulus (NC_020877)</td>
<td>100</td>
<td>42.5</td>
<td>44.8</td>
<td>43.3</td>
<td>43.1</td>
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<tr>
<td>Stab21 (LR215719*)</td>
<td>100</td>
<td>87.4</td>
<td>91.1</td>
<td>92.4</td>
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<tr>
<td>Sb-1 (NC_023009)</td>
<td>100</td>
<td>95.4</td>
<td>96.8</td>
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<tr>
<td>Intesti13</td>
<td>100</td>
<td>96.8</td>
<td></td>
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<tr>
<td>K (NC_005880*)</td>
<td>100</td>
<td>92.2</td>
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Percent genome similarity of five phages (constructed in VIRIDIC using single genome copies)

Supported by NIAID R21 AI163726
Research in Progress
Phage Growth Suppression of MRSA (DNS-VISA)

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<tr>
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<th>8015</th>
<th>306</th>
<th>D712</th>
<th>ATCC 19685</th>
<th>No bact</th>
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<td>Approx. PFU:CFU</td>
<td>1:100</td>
<td>1:100</td>
<td>1:100</td>
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<td>phage-free</td>
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<td>controls</td>
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<td>Romulus + Sb-1</td>
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<td>+ Intesti13</td>
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<td>Romulus</td>
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<td>Stab21</td>
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<tr>
<td>Intesti13</td>
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<td>Sb-1</td>
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<td>K</td>
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Phage activity assessed by bacterial population suppression in broth. PFU:CFU ratios are as plate inoculation.

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Preliminary Time Kill Analysis

MRSA C4: DAP MIC=4, VAN MIC =2, CPT = 0.5 mg/L

Phage: Sb-1, Intesti13

Bacterial quantification in 24 TKA of DAP and CPT (each 0.5 x MIC) combined with phages Intesti13 and Sb-1 at varying MOI against DNS MRSA strain C4

P values determined with one-way ANOVA and Tukey’s Post hoc test. *, P<0.05

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Research in Progress
Phage Cocktail Evaluation: SEV PK/PD Model

MRSA C4: DAP MIC=4, VAN MIC =2, CPT =0.5 mg/L
Phage: Sb-1, Intesti13
Medical Device Infections (MDI) and Impact of Bacterial Embedded Biofilm

• MDIs associated with substantial morbidity and significant healthcare expenditures
• \textit{S. aureus} and coagulase-negative staphylococci are most common pathogens
• Bacterial embedded biofilms significantly reduce antibiotic activity

Live-dead staining of \textit{S. aureus} embedded biofilm
## Phage Activity Against S. aureus Biofilm

### MRSA strains with varying DAP, VAN and CPT Susceptibility

<table>
<thead>
<tr>
<th>Strain</th>
<th>D712</th>
<th>8014</th>
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</thead>
<tbody>
<tr>
<td>Antibiotic</td>
<td>MIC (mg/L)</td>
<td>MBMIC (mg/L)</td>
</tr>
<tr>
<td>DAP</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>VAN</td>
<td>4</td>
<td>8</td>
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<tr>
<td>CPT</td>
<td>0.5</td>
<td>4</td>
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</tbody>
</table>

Biofilm formation by three strain pairs, relative to S. aureus 3678 (reference biofilm strain ATCC35556) positive control and media only (MC) negative control

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CDC Biofilm Reactor Model

Phage Cocktail Activity Against D712 Biofilm

VAN BMIC = 8, DAP BMIC = 8, CPT BMIC = 4 mg/L
Cationic antimicrobial host defense peptides

Figure 1. Examples of cationic antimicrobial host defense peptides. Abbreviations: hBD-1, human beta-defensin-1; hNP-1, human neutrophil peptide-1; mprF, multiple peptide resistance factor; tPMP, thrombin-induced platelet microbicidal protein.

Human Cathelicidin LL-37 Resistance and Increased Daptomycin MIC in Methicillin-Resistant Staphylococcus aureus Strain USA600 (ST45) Are Associated with Increased Mortality in a Hospital Setting

George Sakoulas, a Kripa Guram, a Katherine Reyes, b Victor Nizet, a Marcus Zervos b
University of California San Diego School of Medicine, La Jolla, California, USA a; Henry Ford Hospital, Wayne State University School of Medicine, Detroit, Michigan, USA b

Bacteremia caused by methicillin-resistant Staphylococcus aureus (MRSA) USA600 has been associated with increased patient mortality. We found that USA600 MRSA exhibited significantly increased resistance to human cathelicidin LL-37 killing and daptomycin MIC creep compared to non-USA600 MRSA. Virulent health care-associated MRSA strains may coevolve innate host defense peptide and antibiotic resistances.
Impact of Phage on Innate Immune Factors

LL-37 Survival Time Kill Assay
8014 vs LL-37 4μM + Sb1
Starting inoculum = $10^6$, 10% LB:RPMI used

VAN MIC = 1, DAP = 2 mg/L, OX MIC = > 64 mg/L
In Summary

- Phage therapy continues to evolve
- Many therapeutic questions remain
- Majority of experience is compassionate use
- Empiric versus individualized therapy
- Role of phage-antibiotic combinations
- Standardization is needed for clinical trials